# Germline Variants and Advanced Colorectal Adenomas

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# Germline Variants and Advanced Colorectal Adenomas: Adenoma Prevention with Celecoxib Trial Genomewide Association Study

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## Abstract

**Purpose—**Identification of single nucleotide polymorphisms (SNPs) associated with development of advanced colorectal adenomas.

**Experimental Design—**Discovery Phase: 1,406 Caucasian patients (139 advanced adenoma cases and 1,267 controls) from the Adenoma Prevention with Celecoxib (APC) trial were included in a genome-wide association study (GWAS) to identify variants associated with post-polypectomy disease recurrence. Genome-wide significance was defined as false discovery rate < 0.05, unadjusted $p=7.4\times10^{-7}$. Validation Phase: Results were further evaluated using 4,175 familial colorectal adenoma or CRC cases and 5,036 controls from patients of European ancestry (COloRectal Gene Identification consortium, Scotland, Australia and VQ58).

**Results—**Our study identified eight SNPs associated with advanced adenoma risk in the APC trial (rs2837156, rs7278863, rs2837237, rs2837241, rs2837254, rs741864 at 21q22.2, and rs1381392 and rs17651822 at 3p24.1, at p<10–7 level with odds ratio – OR>2). Five variants in strong pairwise linkage disequilbrium (rs7278863, rs2837237, rs741864, rs741864 and rs2837241, r2=0.8–1) are in or near the coding region for the tight junction adhesion protein, IGSF5. An additional variant associated with advanced adenomas, rs1535989 (minor allele frequency 0.11; OR 2.09; 95% confidence interval 1.50–2.91), also predicted CRC development in a validation analysis (p=0.019) using a series of adenoma cases or CRC (CORGI study) and 3 sets of CRC cases and controls (Scotland, VQ58 and Australia, N=9,211).

**Conclusions—**Our results suggest that common polymorphisms contribute to the risk of developing advanced adenomas and might also contribute to the risk of developing CRC. The variant at rs1535989 may identify patients whose risk for neoplasia warrants increased colonoscopic surveillance.



### Keywords

Colorectal adenomas; colorectal cancer screening; genetic predisposition


## INTRODUCTION

Colorectal cancer (CRC) is a common malignancy, with a prevalence in developed nations of 40–50 cases per 100,000 individuals (1). Approximately one third of those diagnosed with CRC will die of their disease due to diagnosis at a stage not curable by locoregional therapy. Most CRC cases arise from premalignant adenomas that require years or even decades to progress to invasive disease. Colonoscopy to identify and remove precursor adenomas has been recommended for more than 25 years for patients at high CRC risk, and recently completed long-term analyses of screened cohorts confirmed the utility of adenoma removal for preventing deaths due to CRC (2).

Our goal is to understand the biology of CRC in order to develop effective prevention and therapy, and also to characterize individual risk in a manner that will identify patients most likely to benefit from colonoscopy to detect and remove premalignant adenomas. Identification of germline variants conveying an increased risk of CRC could be used to promote adherence to colonoscopy and polypectomy for patients at highest risk, improving

utilization and cost-benefit of this life-saving procedure. In addition, more accurate characterization of high risk individuals would facilitate participation in prevention clinical trials. Finally, therapy to treat or prevent CRC would be advanced if the biological consequences of germline susceptibility variants were further characterized to uncover the molecular basis of CRC.

The adenoma-carcinoma sequence in the colorectum represents a disease spectrum. Adenomas with a low risk of cancer development are small (< 0.6 cm diameter) and lack histological features associated with progression, such as the presence of villous features or high grade dysplasia. The identification of an advanced adenoma (size ≥ 1 cm, villous or tubulovillous histology, high grade dysplasia) indicates that a patient has a higher risk of future adenoma and CRC development (3). Advanced adenomas, therefore, are the most important lesions to target for CRC prevention. In this study, we used a large cohort of adenoma patients from a prospective randomized clinical trial to identify SNPs associated with increased risk of developing advanced adenomas. These variants were then further tested using large genotyped cohorts of patients and controls with advanced adenomas and CRC. In doing so, we identified variants associated with both advanced pre-malignant lesions and CRC.

## METHODS

### Study design and populations (Figure 1)

**Discovery Phase—**1,406 evaluable Caucasian patients were identified from the APC trial, a randomized, placebo-controlled study to test whether celecoxib reduced the occurrence of endoscopically detected colorectal adenomas. The endpoint advanced adenoma was defined as any adenoma with size ≥1cm, villous/tubulovillous histology, or high grade dysplasia. During the prospective follow-up period, 139 participants developed advanced adenomas identified during a scheduled colonoscopy screening exam. Detailed information regarding the trial design and primary outcomes was reported elsewhere (4).

**Validation Phase—**The advanced adenoma susceptibility SNPs identified from APC trial were further evaluated using GWAS data from the following four non-overlapping colorectal cancer case-control series of European ancestry (5).

**(1)** CORGI: 931 familial colorectal adenoma or colorectal cancer cases and 929 cancer-free controls of white British origin ascertained through the COloRectal Gene Identification (CORGI) consortium. All cases had at least one first-degree relative with colorectal tumors and no mutations in the known highly-penetrant CRC genes. Controls were spouses or partners of the cases and had no personal history of CRC(6).

**(2)** Scotland: 1003 early-onset Scottish CRC cases (<55 years) and 979 cancer-free Scottish population controls. Known Mendelian syndromes were excluded. Controls were matched by age (± 5 years), gender and area of residence(6).

**(3)** VQ58: 1,800 British Stage II/III CRC patients from the VICTOR (N=923) and QUASAR2 (http://www.octo-oxford.org.uk/alltrials/trials/q2.html, N=877)

clinical trials, together with publicly available data from 2,690 population controls from the Wellcome Trust Case Control Consortium (WTCCC) 1958 Birth Cohort (7).

**(4)** Australia: 441 CRC cases treated in the Royal Melbourne, Western and St Francis Xavier Cabrini Hospitals in Melbourne and 438 population controls from Brisbane Twin Nevus and Genes in Myopia studies, matched to the cases using principal component analysis(6).

Thus, 4,175 familial colorectal adenoma or CRC cases and 5,036 controls were included in the validation analysis. Human Subjects Committee approval to collect and genotype whole blood samples was obtained by Brigham and Women's Hospital and the RIKEN Center for Genomic Medicine.

## Genotyping and quality control

DNA was isolated from blood samples using standard methods and quantified with picogreen. For the APC cohort, genotyping was performed by the RIKEN Center for Genomic Medicine using the Illumina Human610-Quad BeadChip platform (Illumina, San Diego, CA). A white parent-child CEPH trio from the HapMap was used to check for Mendelian transmission of alleles. Chi-square test based on genotype frequencies at each SNP was used to test for deviations from Hardy-Weinberg equilibrium (HWE). Any SNP with HWE $p<0.001$ was excluded. Two cases and two controls were randomly chosen as duplicates for quality control (QC) of genotype concordance. A total of 28 subjects and 1,792 markers were excluded for quality control reasons, including duplicates, those that showed identity-by-descent >12.5% or were gender mismatched, samples with <98% and markers with <99% call rate or heretozygous haploids. The final Manhattan plot and QQ plot indicated the satisfactory QC process (supplement Figure 1 and 2).

For the additional susceptibility evaluation cohorts, samples were genotyped on Illumina Infinium SNP arrays, ranging from the Hap300 (for VQ58) to the Hap1M (for Australia). Details concerning genotyping and quality control for these studies have been provided previously (5). Ethics Committees approved these five studies and samples were collected in accordance with the tenets of the Declaration of Helsinki.

Among the top 19 SNPs identified from the APC trial, 12 SNPs had genotype data available from the CORGI, Scotland, VQ58 or Australia GWA studies. Nine of these SNPs were typed in all four studies (rs1381392, rs17651822, rs17781398, rs16909065, rs9582985, rs2837156, rs2837241, rs741864) and three were typed in three (rs13085889, rs1424593 and rs2837237, Supplemental Table 1).

## Statistical methods

To assess the strength of association between genotype and advanced adenoma risk, a per allele unconditional logistic regression model was used to estimate odd ratios (ORs) and their corresponding 95% confidence intervals (CIs). For the APC trial, genotype-phenotype interactions were evaluated for sex, age at trial entry (≤ age 60 years vs. > age 60 years), and family history (first-degree relatives with colorectal cancer). Genotype-environment

interactions were evaluated for aspirin use at baseline and treatment with celecoxib. The Breslow-Day test was used to test the homogeneity of odds ratios. PLINK (http://pngu.mgh.harvard.edu/~purcell/plink/), STATA and SAS were used to conduct all the analysis. Genome-wide significance was defined as false discovery rate (FDR) < 0.05, which corresponds to an unadjusted $p=7.4\times10^{-7}$ in this analysis(8, 9).

## RESULTS

1,406 evaluable Caucasian patients were genotyped from the APC trial (4). Eight SNPs were identified by association with on-study development of advanced adenomas at a genome-wide level of significance: rs2837156, rs7278863, rs2837237, rs2837241, rs2837254, rs741864, all at 21q22.2, and rs1381392 and rs17651822, both at 3p24.1 (Table 1). The associations between the 6 SNPs in the 21q22.2 region and advanced adenoma development were all highly significant (unadjusted $p=10^{-8}$–$10^{-9}$) with ORs per allele ranging from 2.22 to 2.55. All 6 SNPs in the 21q22.2 region were located near the coding region for the adherens junction protein, *IGSF5*, and five of these SNPs (rs7278863, rs2837237, rs741864, rs741864 and rs2837241) were in strong linkage disequilibrium ($r^2=0.8$–1, Figure 1). For the 3p24.1 signal, the OR for genotype rs1381392 was 2.01 (95% CI 1.52–2.65, unadjusted $p=7.4\times10^{-07}$), and that for rs17651822 was 2.16 (95% CI 1.61–2.91, unadjusted $p=2.1\times10^{-07}$).

Eleven SNPs (rs11886781 at 2p24.2, rs13085889 at 3q22.2, rs1424593, rs1364512 and rs7778725 at 7q32.3, rs16909065 and rs16909036 at 9q33.2, rs17654765, rs1535989 and rs9582985 at 13q33.2) were associated with moderate (~2-fold) ORs for advanced adenoma detection, but the associations did not reach genome-wide significance ($p \le 10^{-6}$). Of these 11 SNPs, 6 mapped to gene coding regions: rs11886781 to *KCNS3*, rs17781398 to *FAM188b*, rs13085889 to *EPHB1* and *KY*, and rs1424593, rs1364512 and rs7778725 all to *PLXNA4* (Table 1 and Figure 1).

There are no comparable adenoma chemoprevention cohorts currently available for validation of the APC GWAS results. We therefore further examined APC trial results using GWAS data from four non-overlapping CRC case-control series of European ancestry, one of which (CORGI) also included advanced adenoma cases (5). Among the 19 advanced adenoma risk SNPs with a nominal significance level of $p\le10^{-6}$, 12 were genotyped in at least 3 of the available four CRC GWA studies (Supplemental Table 1). Allelic frequencies of each variant and the corresponding associations with CRC phenotype were accessed in each of the 4 case-control samples. The results of the meta-analysis for overall associations with CRC risk are reported in Table 2 and supplement figure 3.

One of the 19 SNPs identified in the APC trial, rs1535989, was replicated in the independent CRC cohorts, with an OR for CRC development of 1.12 (95% CI 1.019–1.23, p=0.019). There was no evidence of inter-study heterogeneity (Phet =0.71; I2=0.0%). An additional exploratory meta-analysis was performed, combining all five studies and using either advanced adenoma or CRC as the outcome (Table 2). SNP rs9582985 originally identified in the APC cohort showed marginally significant association with outcome (OR=1.11, p=0.055).

Clinical data from the APC trial was used to further characterize rs1535989 by examining the association of this variant with other susceptibility factors for advanced colorectal neoplasia, including age, sex, aspirin use at baseline, family history of CRC and on-study treatment with celecoxib. SNP and environmental factors interaction terms were included in the model. SNP rs1535989 showed statistically significant interactions with subjects' age ($p=0.0016$), sex ($p=0.0057$) and aspirin use at baseline ($p=0.02$). The associations with advanced neoplasia were stronger in older individuals (>60, OR 3.20; 95% CI 2.10–4.87), males (OR 2.74; 95% CI 1.89–3.97), and those using aspirin at baseline (OR=3.63; 95% CI 2.06–6.40) (Table 3). There were no statistically significant interactions with CRC family history or on-study treatment with celecoxib.

## DISCUSSION

Among the approximately 145,000 CRC cases diagnosed per year in the United States, only 5% represent autosomal dominant predisposition syndromes, with the majority of these involving either hereditary nonpolyposis colon cancer (HNPCC) or familial adenomatous polyposis (FAP). An additional 20–25% of CRC cases show a familial association without precise genetic characterization, and the majority of CRCs occur in individuals without a family history of the disease. Self-reported family history does not accurately assess the inherited risk of advanced adenomas because patients' knowledge of their family history of colorectal adenomas is often unknown or incomplete (10). Recent GWAS from members of this collaboration have identified 18 CRC susceptibility variants with minor allele frequencies ranging from 0.07 to 0.48 that each convey a small degree of risk modification (OR per allele: 0.87–1.35) (11–15). The results presented here expand these data to address inherited susceptibility for developing advanced adenomas that represent targets for CRC prevention. In addition to the studies whose data were used here, there have been a number of other GWAS with CRC or colorectal adenomas as the primary phenotype (16–20). These have yielded a substantial number of possible susceptibility variants, most conveying modestly altered risk. A recent case-control meta-analysis from 14 studies identified SNPs on 2q32.3 (rs11903757), 1q25.3 (rs10911251), 12p13.32 (rs3217810), and 12q24.21 (rs59336) that represented odds ratios ranging from 0.84–1.15 (16).

The APC trial was designed to determine whether the selective cyclooxygenase-2 inhibitor, celecoxib, prevented adenomas in patients at high risk for CRC. Eligibility criteria required that participants have at least one prior adenoma > 6mm in size, or multiple adenomas. During the 5 years of endoscopic surveillance, 21.3% of APC trial participants randomized to placebo developed recurrent advanced adenomas (21). This rate was decreased to 12.5% in patients receiving celecoxib 200 mg twice daily ($p<0.0001$), however concerns over cardiovascular toxicity currently prohibit the use of celecoxib for routine CRC chemoprevention (22). Results presented here showed that advanced adenomas were twice as likely to occur in APC trial participants with variant rs1535989, and that this increased risk was not affected by celecoxib treatment. For males or older individuals, the risk was more than 3-fold higher than that for females or participants <age 60. The observed interaction between baseline aspirin use and advanced adenoma risk is particularly interesting. Aspirin use reduces the incidence of colorectal adenomas and CRC, and subjects enrolled in the APC trial who used aspirin at baseline were those who developed adenomas

despite aspirin use. These individuals may therefore have constituted a higher risk subset because they were relatively resistant to aspirin chemoprevention. The analyses conducted here showed that APC trial participants who both developed adenomas while taking aspirin and had variant rs1535989 demonstrated a 3.63-fold increase in advanced adenoma risk during surveillance. If this association can be confirmed in other studies, and other variants of similar effect found, then genotyping will represent a useful method to target high risk patients for preventive treatments including more frequent colonoscopic screening.

Additional results from this GWAS suggest areas for further research concerning the molecular basis of colorectal neoplasia. Variants at rs2837156, rs7278863, rs2837237, rs2837241, rs2837254 and rs741864 are in close association at 21q22.2. Two of these SNPs, rs2837156 and rs7278863, are within the coding region of *IGSF5*, a gene encoding a transmembrane protein whose murine homologue, JAM4, binds to the tumor suppressor, MAGI-1 at intestinal epithelial tight junctions (23). To explore the potential effect of IGSF5 on the prognosis of CRC patients, microarray expression data from the GEO data set (GSE14333) were retrieved for 229 Dukes A, B, and C patients (24). The gene expression profile was performed with Affymetrix u133p2 platform. Our preliminary analysis of these data indicated that the overexpression of IGSF5 is associated with significantly worse relapse-free survival (unadjusted p=0.000004, bonferroni adjusted p=0.00085, supplement figure 4). In addition, rs1424593, rs1364512 and rs7778725 all involve *PLXNA4*, a member of the plexin family located on chromosome 7. Plexins are transmembrane, secreted, and GPI-anchored semaphorins that modulate the adhesive and migratory properties of malignant cells. The protein product of *PLXNA4* forms stable complexes with FGFR1 and VEGFR-2 tyrosine kinase receptors and enhances both VEGF-induced VEGFR-2 phosphorylation and βFGF-induced cell proliferation (25). Finally, *EPHB1* encodes a ligand that binds to an Eph receptor tyrosine kinase to mediate bidirectional signaling required for intestinal epithelial homeostasis (26). EphB-ephrin B interactions regulate cell adhesion, migration and positioning, and play an important role in colorectal tumor progression (27).

The limitation of our current study resides in the following two aspects. The limited number of advanced adenoma cases in the APC trial restricted the power to identify more advanced adenoma susceptibility SNPs. In addition, the CRC/adenoma cases with a strong family history of CRC were not excluded from replication datasets. This might under/overestimate the association between identified SNPs and sporadic CRC risks.

In summary, this study identified 19 SNPs associated with advanced adenoma risk at a level of $p \leq 10^{-6}$. Of these, 12 SNPs were tested in a meta-analysis using independent datasets to evaluate their association with CRC development, and rs1535989 was also associated with increased risk of both advanced adenomas and CRC. In addition, eight of the variants identified in the APC trial mapped to coding regions of genes previously implicated in CRC progression, and warrant further study to confirm their role in modifying tissue-specific biological function.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Abbreviations

| | |
|---|---|
| **SNP** | Single nucleotide polymorphism |
| **CRC** | Colorectal cancer |
| **APC** | Adenoma Prevention with Celecoxib study |
| **GWAS** | Genome-wide Association Study |
| **Corgi** | Colorectal gene identification study |
| **FAP** | Familial adenomatous polyposis |
| **NBS** | National blood service |
| **NPS** | National Polyp Study |

| | |
|---|---|
| **OR** | odds ratio |
| **BC58** | 1958 Birth Cohort. |

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## STATEMENT OF TRANSLATIONAL RELEVANCE

Identification of patients at highest risk of colorectal cancer is essential for providing optimal disease screening and prevention. This study uncovers germline susceptibility loci that indicate risk of disease and potential for improved understanding of disease biology.

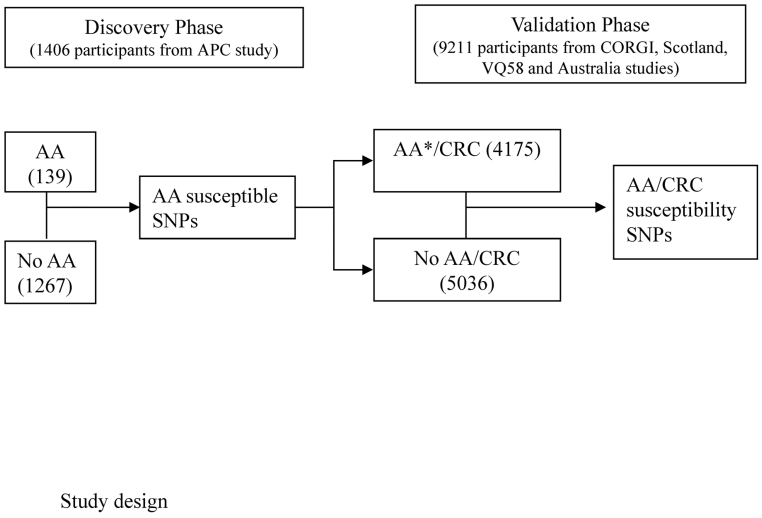
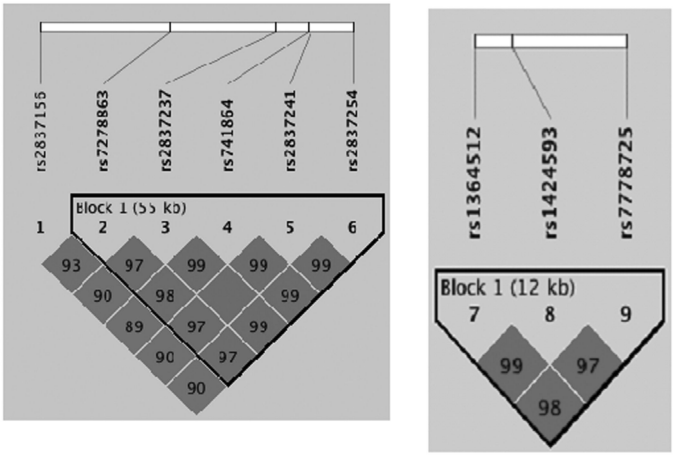



**Figure 1.**

AA: advanced adenoma *Including subjects with familial adenoma from CORGI




| **21q22.2** | rs2837156 | rs7278863 | rs2837237 | rs2837241 | rs2837254 | rs741864 |
|---|---|---|---|---|---|---|
| rs2837156 | 1 | <0.8 | <0.8 | <0.8 | <0.8 | <0.8 |
| rs7278863 | | 1 | 0.84 | <0.8 | <0.8 | 0.93 |
| rs2837237 | | | 1 | 1 | 0.89 | 0.9 |
| rs2837241 | | | | 1 | 0.89 | 0.9 |
| rs2837254 | | | | | 1 | 0.97 |
| rs741864 | | | | | | 1 |

| **7q32.3** | rs142593 | rs1364512 | rs7778725 |
|---|---|---|---|
| rs142593 | 1 | 0.97 | 0.92 |
| rs1364512 | | 1 | 0.94 |
| rs7778725 | | | 1 |

LD analysis of SNPs on 21q22.2 and 7q32.3 by pairwise $r^2$

**Figure 2.**
The 6 IGSF-5 related SNPs are within very tight LD region

## Table 1

APC trial advanced adenoma susceptibility loci*

| Chromosome Region | SNP | Position (BP) | Alleles | | MAF | p value | OR | Gene |
|---|---|---|---|---|---|---|---|---|
| 2p24.2 | rs11886781 | 18154780 | A | C | 0.08 | 9.7E-06 | 2.25(1.56,3.25) | KCNS3 EPHB1, |
| 3q22.2 | rs13085889 | 135843760 | A | C | 0.29 | 8.8E-06 | 1.77(1.37,2.29) | KY |
| 3p24.1 | rs1381392 | 28724318 | A | G | 0.18 | 7.4E-07 | 2.01(1.52,2.65) | |
| 3p24.1 | rs17651822 | 28695130 | A | G | 0.14 | 2.1E-07 | 2.16(1.61,2.91) | |
| 7p14.3 | rs17781398 | 30807966 | A | G | 0.10 | 9.0E-06 | 0.19(0.08,0.43) | FAM188b |
| 7q32.3 | rs1424593 | 131605541 | C | A | 0.50 | 9.1E-06 | 0.56 (0.44, 0.73) | PLXNA4 |
| 7q32.3 | rs1364512 | 131602384 | C | A | 0.49 | 8.6E-06 | 0.56(0.42,0.71) | PLXNA4 |
| 7q32.3 | rs7778725 | 131614936 | G | A | 0.49 | 4.0E-06 | 0.55(0.42,0.71) | PLXNA4 |
| 9q33.2 | rs16909065 | 121597606 | A | G | 0.05 | 3.6E-06 | 2.59(1.71,3.93) | |
| 9q33.2 | rs16909036 | 121587049 | G | A | 0.05 | 3.7E-06 | 2.59(1.71,3.93) | |
| 13q33.2 | rs1535989 | 104820723 | G | A | 0.11 | 8.9E-06 | 2.09(1.50,2.91) | |
| 13q33.2 | rs17654765 | 104828038 | A | G | 0.10 | 4.7E-06 | 2.14(1.53,2.98) | |
| 13q33.2 | rs9582985 | 104829133 | C | A | 0.11 | 9.3E-06 | 2.05(1.48,2.83) | |
| 21q22.2 | rs2837156 | 40048557 | G | A | 0.12 | 3.2E-07 | 2.22(1.62,3.03) | IGSF5 |
| 21q22.2 | rs7278863 | 40087578 | A | G | 0.10 | 1.4E-08 | 2.48(1.80,3.42) | IGSF5 |
| 21q22.2 | rs2837237 | 40119727 | G | A | 0.12 | 3.6E-09 | 2.48(1.82,3.38) | |
| 21q22.2 | rs2837241 | 40130476 | A | C | 0.12 | 3.7E-09 | 2.48(1.82,3.38) | |
| 21q22.2 | rs2837254 | 40143171 | A | G | 0.11 | 2.9E-09 | 2.55(1.86,3.51) | |
| 21q22.2 | rs741864 | 40129665 | A | G | 0.11 | 1.1E-08 | 2.48(1.80,3.41) | |

* Total number of subjects is 1,406, of which 139 developed advanced adenomas

**Table 2**

Meta-analysis using adenoma or CRC as a composite outcome

| SNP | N | P | P(R) | OR | OR(R) | Q | I |
|---|---|---|---|---|---|---|---|
| rs13085889 | 4 | 0.1048 | 0.1048 | 1.0575 | 1.0575 | 0.5438 | 0 |
| rs1381392 | 4 | 0.4636 | 0.4636 | 1.0295 | 1.0295 | 0.4186 | 0 |
| rs1424593 | 3 | 0.405 | 0.405 | 1.027 | 1.027 | 0.4233 | 0 |
| rs1535989 | 4 | **0.012** | 0.012 | 1.1304 | 1.1304 | 0.7925 | 0 |
| rs 16909065 | 4 | 0.1273 | 0.1273 | 0.9054 | 0.9054 | 0.498 | 0 |
| rs17651822 | 4 | 0.689 | 0.689 | 1.0176 | 1.0176 | 0.8499 | 0 |
| rs17781398 | 4 | 0.7972 | 0.7972 | 1.016 | 1.016 | 0.9487 | 0 |
| rs2837156 | 4 | 0.8017 | 0.8017 | 0.9881 | 0.9881 | 0.4326 | 0 |
| rs2837210 | 4 | 0.8706 | 0.9766 | 1.0083 | 0.9983 | 0.3114 | 16.05 |
| rs2837237 | 3 | 0.3464 | 0.3464 | 0.9379 | 0.9379 | 0.413 | 0 |
| rs2837241 | 4 | 0.938 | 0.8585 | 0.9963 | 0.9907 | 0.3395 | 10.69 |
| rs741864 | 4 | 0.6248 | 0.6248 | 0.971 | 0.971 | 0.6068 | 0 |
| rs9582985 | 4 | 0.05468 | 0.05468 | 1.1188 | 1.1188 | 0.9416 | 0 |

## Table 3

Genotype-phenotype/environment interactions for SNP rs1535989

| Phenotype | | OR | Interaction (p value) |
|---|---|---|---|
| Age | ≤60 | 0.91(0.42, 1.76) | |
| | >60 | 3.20(2.10,4.87) | 0.0016 |
| Sex | Female | 0.65(0.25, 1.68) | |
| | Male | 2.74(1.89,3.97) | 0.0057 |
| Family History | No | 2.23(1.51,3.28) | |
| | Yes | 1.74(0.88,3.43) | 0.53 |
| Prior Aspirin | No | 1.58(1.03,2.42) | |
| Use | Yes | 3.63(2.06, 6.40) | 0.02 |
| Treatment | Placebo | 1.63(1.03,2.58) | |
| | 200mg | 2.51(1.27,4.95) | |
| | 400mg | 2.43(1.16,5.07) | NS |